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# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: BHV-314.01

U S APPLICATION NO. (If known, see 37 CFR 1.5): 09/424498

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
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| PCT/EP98/03090 | 26 .05.98)<br>26 May 1998 | (28.05.97)<br>28 May 1997 |

TITLE OF INVENTION: PHARMACEUTICAL PREPARATION COMPRISING vWF PROPEPTIDE

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is the FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ( ) This a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. (X) This express request to begin national examination procedures (35 U.S.C. 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. (X) A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. (X) A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. (X ) is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ( ) has been transmitted by the International Bureau.
   c. ( ) is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ( ) A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ( ) Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ( ) are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ( ) has been transmitted by the International Bureau.
   c. ( ) have not been made; however, the time limit for making such amendments has NOT expired.
   d. ( ) have not been made and will not be made.
8. ( ) A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. (X) An unexecuted oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ( ) A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**Items 11. to 16. below concern document(s) or information included:**

11. ( ) An information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ( ) An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. (X) A FIRST preliminary amendment.
    ( ) A SECOND or SUBSEQUENT preliminary amendment.
14. ( ) A substitute specification.
15. ( ) A change of power of attorney and/or address letter.
16. (X) Other items of information.
    (X) Figures 1-4
    (X) Copy of PCT Request Form
    (X) Copy of Cover Sheet of international publication no. WO98/53848
    (X) Copy of International Preliminary Examination Report
    (X) Copy of International Search Report
    (X) Copy of Form PCT/IB/306 (Notification of the Recording of a Change - New Assignee is BAXTER AKTIENGESELLSCHAFT)
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Carmen Parra

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| U.S. APPLICATION NO (if known, see 37 CFR 1.5) | INTERNATIONAL APPLICATION NO | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| 09/424498 | PCT/EP98/03090 | BHV-314.01 |

17. (x) The following fees are submitted:

| BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)): | | CALCULATIONS PTO USE ONLY |
|---|---|---|
| Search Report has been prepared by the EPO or JPO | $840.00 | |
| International preliminary examination fee paid to USPTO (37 CFR 1.482) | $670.00 | |
| No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) | $760.00 | |
| Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO | $970.00 | |
| International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) | $96.00 | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | | $ 970.00 |
| Surcharge of **$130.00** for furnishing the oath or declaration later than months from the earliest claimed priority date (37 CFR 1.492(e)). | ( ) 20 (x) 30 | $0 |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | |
|---|---|---|---|---|
| Total claims | 33 - 20 = | 13 | X $18.00 | $ 234.00 |
| Independent claims | 6 - 3 = | 3 | X $78.00 | $ 234.00 |
| MULTIPLE DEPENDENT CLAIM(S) (if applicable) | | | + $260.00 | $0 |
| | **TOTAL OF ABOVE CALCULATIONS =** | | | $1438.00 |
| Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). | | | | $0 |
| | **SUBTOTAL =** | | | $1438.00 |
| Processing fee of **$130.00** for furnishing the English translation later than months from the earliest claimed priority date (37 CFR 1.492(f)). | ( ) 20 ( ) 30 | | + | $0 |
| | **TOTAL NATIONAL FEE =** | | | $1438.00 |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property. | | | + | $0 |
| | **TOTAL FEES ENCLOSED =** | | | $1438.00 |
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a. ( X) A check in the amount of $1438.00 to cover the above fees is enclosed.

b. ( ) Please charge my Deposit Account No **06-1448** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. (X) The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **06-1448, (Ref. BHV-314.01).** A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

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Boston, MA 02109-2170

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Beth E. Arnold
REGISTRATION NO. 35,430



373525

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re the application of:
Schwarz et al.

U.S. Application Serial No.: To Be Determined;
National Phase of PCT/EP98/03090
Filed May 26, 1998 (Priority Date: May 28, 1997)

For: *Pharmaceutical Preparation Comprising vWF Propeptide*

Filed: November 24, 1999

Attorney Docket No.:
**BHV-314.01**

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November 24, 1999
Date of Signature and of Mail Deposit

By: Carmen Parra
Carmen Parra

**PRELIMINARY AMENDMENT**

Dear Sir:

Please amend the above-identified patent application as follows:

In the claims:

Cancel claims 1-30 and add new claims 31-63 as follows:

-- 31. A pharmaceutical preparation for treating blood coagulation disorders, said preparation comprising an effective amount of vWF propeptide.

32. A preparation as set forth in claim 31, said preparation being essentially comprised of vWF propeptide.

33. A preparation as set forth in claim 31, comprising pro-vWF, said pro-vWF containing said vWF propeptide.

34. A preparation as set forth in claim 33, wherein said pro-vWF is a recombinant pro-vWF.

35. A preparation as set forth in claim 31, further comprising a hemostasis protein.

36. A preparation as set forth in claim 35, wherein said hemostasis protein is a blood factor.

37. A preparation as set forth in claim 36, wherein said blood factor is selected from the group consisting of mature vWF, factor VIII, activated blood coagulation factors, blood factors with FEIB activity and FEIBA.

38. A preparation as set forth in claim 33, further comprising factor VIII, said pro-vWF being complexed to said factor VIII.

39. A preparation as set forth in claim 31, further comprising a platelet component.

40. A preparation as set forth in claim 39, wherein said platelet component is at least one component selected from the group consisting of collagen, a platelet glycoprotein, a platelet, fibrinogen, fibrin, heparin and a derivative thereof.

41. A preparation as set forth in claim 31, further comprising phospholipids.

42. A preparation as set forth in claim 31, said preparation having been treated for at least one of virus inactivation and virus removal.

43. A preparation as set forth in claim 31, further comprising a pharmaceutically acceptable carrier.

44. A preparation as set forth in claim 31, wherein said vWF propeptide is a recombinant vWF propeptide.

45. A method for producing a pharmaceutical preparation containing an effective amount of vWF propeptide, said method comprising providing a source material containing said vWF propeptide, separating said vWF propeptide from said source material, and formulating said vWF propeptide to a pharmaceutical preparation.

46. A method as set forth in claim 45, further comprising subjecting said vWF propeptide to at least one of a virus inactivation and a virus removing treatment.

47. A method as set forth in claim 45, wherein said source material is selected from the group consisting of plasma and a plasma fraction.

48. A method as set forth in claim 45, wherein said source material is obtained from a cell culture.

49. A method as set forth in claim 45, wherein said vWF propeptide is produced by recombinant DNA technology.

50. A method as set forth in claim 45, wherein said vWF propeptide is contained in a pro-vWF.

51. A method as set forth in claim 50, wherein said pro-vWF is a mutant pro-vWF with a mutation at the cleavage site of the vWF propeptide.

52. A method as set forth in claim 50, further comprising providing an inhibitor inhibiting cleavage of said vWF propeptide from said pro-vWF, said pharmaceutical preparation being produced in the presence of said inhibitor.

53. A method as set forth in claim 45, wherein said vWF propeptide is separated from said source material by chromatography.

54. A method as set forth in claim 53, wherein said chromatography is an affinity chromatography.

55. A method as set forth in claim 54, further comprising using carrier materials with ligands specific for said vWF propeptide for said affinity chromatography.

56. A method for treating a patient running a risk of a blood coagulation disorder comprising administering an effective dose of a pharmaceutical composition comprising at least one of a vWF propeptide and pro-vWF containing said vWF propeptide to said patient.

57. A method for treating and preventing blood coagulation disorders in a patient, comprising administering to said patient an effective dose of a pharmaceutical composition comprising at least one of a vWF propeptide and a pro-vWF containing said vWF propeptide.

58. A method as set forth in claim 57, wherein said patient is a vWD inhibitor patient.

59. A method of improving the compatibility of pharmaceutical vWF preparations, wherein at least one agent selected from the group consisting of pp-vWF and pro-vWF is administered to a patient when a pharmaceutical vWF preparation is administered to said patient.

60. A method for treating or preventing adverse effects of endogenous or exogenous vWF, wherein a pharmaceutical composition containing one of pp-vWF and pro-vWF is administered to a patient in an effective dose.

61. A method as set forth in claim 60, wherein said adverse effects are selected from the group consisting of elevated vWF levels associated with thrombotic thrombocytopenic purpura, Henoch-Schönlein Purpura, preclampsia, neonatal thrombocytopenia, hemolyticuremic syndrome, myocardial infarction and a poor outcome following arterial surgery.

62. A method as set forth in claim 57, wherein said patient suffers from hemophilia.

63. A method as set forth in claim 52, wherein said hemophilia is selected from the group consisting of phenotypic hemophilia, hemophilia A and factor VIII inhibitors. - -

Applicants submit that the claims being added in the preliminary amendment and the specification are in compliance with all patentability requirements. Applicants therefore respectfully request that the claims be allowed. To expedite allowance, the Examiner is encouraged to contact Applicants' attorney at the number provided below.

Respectfully submitted,
Foley, Hoag & Eliot, LLP

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# Pharmaceutical Preparation Comprising vWF Propeptide

The invention relates to a pharmaceutical preparation comprising the vWF-propeptide (pp-vWF).

Von Willebrand factor (vWF) is a glycoprotein circulating in plasma as a series of multimeres ranging in size from about 500 to 20 000 kD. Multimeric forms of vWF are composed of 250 kD polypeptide subunits linked together by disulfide bonds. vWF mediates the initial platelet adhesion to the sub-endothelium of the damaged vessel wall, only the larger multimers also exhibiting hemostatic activity. It is assumed that endothelial cells secret large polymeric forms of vWF and that those forms of vWF which have a low molecular weight (low molecular weight vWF) have arisen from proteolytic cleavage. The multimers having large molecular masses are stored in the Weibel-Pallade bodies of the endothelial cells and liberated upon stimulation.

vWF is synthesized by endothelial cells and megakaryocytes as prepro-vWF that consists to a large extent of repeated domains. Upon cleavage of the signal peptide pro-vWF dimerizes through disulfide linkages at its C-terminal region. The dimers serve as protomers for multimerization which is governed by disulfide linkages between the free end termini. The assembly to multimers is followed by the protelytic removal of the propeptide (Leyte et al., Biochem.J. 274 (1991), 257-261).

The full length of cDNA of vWF was cloned; the propolypeptide corresponds to amino acid residues 23 to 764 of the full length prepro-vWF (Eikenboom et al (1995) Haemophilia 1, 77-90).

The propeptide of vWF (pp-vWF) was shown to be identical to the von Willebrand antigen II, the second identified antigen that is deficient in the plasma and platelets of patients with severe von Willebrand disease (vWD). pp-vWF is specifically localized in platelets since plasma contains less than 5% of total propeptide vWF in blood, assuming the platelet count is $3 \times 10^8$ per ml. As already known, pp-vWF is released from platelets upon activation by various agonists. The pp-vWF is a glycoprotein not

only because it reacts with periodic acid Schiff's reagent but also because it binds to lentil lectin. pp-vWF binds specifically to native type I collagen, but does not bind to heat-denatured collagen. It was shown that the affinity between pp-vWF and type I collagen was quite high so that the binding - which does not require any divalent cation and is not affected by addition of a peptide that contains sequence of arginine-glycine-aspartic acid (that is known to inhibit many cell attachment processes) - rapidly reached equilibrium.

The physiological role of pp-vWF is postulated to lie in the government of the assembly of vWF multimers, either before or after the cleavage from pro-vWF molecules. (Takagi et al., JBC 264 (18) (1989), 10425-10430).

pp-vWF was also shown to inhibit the platelet collagen interaction action (Takagi et al., JBC 264(11) (1989), 6017-6020).

In Isobe et al. (JBC 272 (13) (1997), 6447-6453) the role of pp-vWF as a novel physiological ligand and an adhesion substrate for $\alpha 4\beta 1$ integrin-expressing leukemia cells was investigated. It was found that pp-vWF plays an important role in the mechanism underlying the melanoma metastasis as well as vascular inflammation.

Although pharmaceutical preparations containing mature vWF are known (see e.g. US 5,571,784) the pharmaceutical usage of pp-vWF or the pro-form vWF have not been described or suggested in the prior art. According to the US 5,571,784 vWF does not impair the systemic anticoagulatory effect of the anticoagulant hirudin as measured by the aPTT, rather it decreases the bleeding side effects of anticoagulant therapy. vWF is therefore proposed as a pseudo-antidote in assocation with hemorrhages which are produced by administering antithrombotic and/or fibrinolytic agents.

From Blann et al. (Eur.J.Vasc.Surg.8 (1994), 10-15) it is also known that vWF levels are increased with risk factors for

atherosclerosis and in patients with diffuse arterial disesase. The level of vWF is also thought to be a measure of endothelial $_4$>integrin-expressing leukemia cells was investigated. It was found that pp-vWF plays an important role in the mechanism underlying the melanoma metastasis as well as vascular inflammation.

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From Blann et al. (Eur.J.Vasc.Surg.8 (1994), 10-15) it is also known that vWF levels are increased with risk factors for atherosclerosis and in patients with diffuse arterial disesase. The level of vWF is also thought to be a measure of endothelial damage in the inflammatory vasculitides. Providing low levels of vWF in a patient is suggested to be protective for such diseases.

It is is the object of the present invention to provide a vWF pharmaceutic with improved properties. The preparation should enhance the intrinsic blood coagulation activity in individuals and reduce the arterial thrombotic risk of vWF therapy.

This object is solved by the present invention by providing a pharmaceutical preparation for treating blood coagulation disorders comprising an effective amount of vWF propeptide. It was found out that pp-vWF plays an essential role in blood coagulation. It promotes the intrinsic blood coagulation and thereby acts on secondary heostasis. At the same time it inhibits the platelet adhesion and controls the primary hemostatic activity of mature vWF by binding to collagen. Based

on these findings, a vWF preparation can be improved providing additional pro-vWF or pp-vWF as a separate effective protein in the vWF preparation. pp-vWF controls the primary hemostatic activity of the mature vWF and thus reduces the potential thrombotic risk of vWF for e.g. inducing arterial thrombosis as indicated by the prior art.

It was surprisingly found out that a recombinant vWF preparation (rvWF) containing substantial amounts of pp-vWF induces an elevated thrombin generation in vitro. The thrombin generation was measured by an in vitro assay performed with washed vWD platelets in FVIIIC substituted vWD plasma upon addition of FEIBA (factor VIII inhibitor bypassing activity; Austrian patents No 350 726, 368 883 and 398 079) to initiate the activation of prothrombin. When the rvWF preparation contained a predominant amount of pro-vWF and pp-vWF the thrombin generation was substantially increased. The contribution of the pp-vWF to the coagulation-promoting effect of vWF is therefore surprisingly significant.

Since these results imply that the effect of vWF as blood coagulation factor is related to pp-vWF, the pharmaceutical preparation based on the pp-vWF or pro-vWF is applicable in areas wherein vWF has been proposed and even more areas where a coagulation-promoting effect is desired.

The preparation of the pp-vWF or pro-vWF is well known in the art due to many papers relating to expression and properties of vWF or for diagnostic purposes, see e.g. experimental procedures in Isobe et al., Takagi et al. (both 1989 papers) or Leyte et al.. However, there was not yet any individual coagulation promoting activity reported to be associated with vWF and the propeptide region of vWF and therefore not a respective pharmaceutical preparation. Since the pp-vWF is a well defined polypeptide and easily prepared by recombinant DNA-technology, the recombinant way of production is a preferred embodiment for producing the present pharmaceutical preparation. The polypeptide may also be purified or purified further by chromatography, such as by ion exchange chromatography and/or

affinity chromatography using monoclonal antibodies, heparin, collagen, factor VIII protein, or fragments thereof as affinity ligands. It is also possible to separate pp-vWF from contaminating proteins and/or mature vWF by gel filtration.

A preferred embodiment of the present invention is a pharmaceutical preparation essentially consisting of the vWF-propeptide. Thereby the preparation contains purified pp-vWF or pro-vWF to the extent that at least 80%, preferably at least 90%, most preferred more than 95% or about 100% pure pp-vWF or pro-vWF is contained.

Another embodiment of the present invention is a preparation essentially consisting of the pro-vWF containing the vWF-propeptide as a pharmaceutical preparation.

As stated above it is preferred to use recombinantly produced pro-vWF or pp-vWF for the present preparation. (FEBS Letters 351 (1994), 345-348 or Blood 88 (8) 19996, 2951-2958)

The preparations of the present invention preferably contain at least 10 nM pp-vWF, more preferably at least 30 nM, especially more than 50 nM, and/or at least 10 nM pro-vWF, more preferably more than 100 nM, especially more than 250 nM. The effective amount is defined to obtain a pp-vWF level of at least twice the physiologic amount in human plasma. In Blood 88 (8) 1996, 2951 - 2958 it is reported that the molar ratio of the propeptide and vWF concentration is about 0,1 in Normal Plasma. The pp-vWF is considered as a rather abundant protein at a concentration of 5 to 100 nmol/L depending on the state of activation of the endothelium.

If vWF is contained in the preparation besides pro-vWF the molar ratio of the pro-vWF and vWF is at least 10%, which may be measured as U-Antigen detected by a polyclonal antibody preparation directed against vWF antigen. In the preferred preparation according to the invention the molar ratio is even higher, at least 20% or more preferred at least 50%. The most effective preparation according to the invention contains more

than 80% of vWF-Antigen as pro-vWF.

It turned out that a pharmaceutical preparation according to the invention based on pro-vWF is rapidly processed upon administration in vivo. The thus generated pp-vWF is effective in its thrombin potential and coagulation-promoting activity.

In another preferred embodiment the pharmaceutical preparation according to the present invention further contains a hemostasis protein, preferably a blood factor. Preferred embodiments of these blood factors are selected from the group consisting of mature vWF, factor VIII, activated blood coagulation factors, blood factors with FEIB-activity and FEIBA. Any hemostasis protein decreasing the aPTT or PT of normal plasma is a suitable combination with the pp-vWF or pro-vWF.

A combination of the pp-vWF preparation with FVIIIC provides for a pharmaceutical preparation with improved coagulation activity. When the propeptide is in the form of pro-vWF that is complexed to FVIIIC, the pharmaceutical preparation according to the present invention shows additionally improved FVIIIC-stability.

The further combinations in a pharmaceutical preparation according to the invention are provided with a platelet component. Some of the components having binding properties or activity to vWF or pro-vWF or pp-vWF, which are suitable to contribute to the physiological activities, are collagen, platelet glycoprotein, a platelet, fibrinogen, fibrin, heparin, or a derivative thereof.

The pharmaceutical preparation of the present invention may also further contain phospholipids.

The pharmaceutical preparation according to the present invention preferably has been made virus safe by treating for virus inactivation or removal.

The virus inactivation or removal treatment may be performed by any treatment accepted as being efficient. According to

preferred embodiments of the present invention the pharmaceutical composition is treated with tensides and/or heat, e.g. by a heat treatment in the solid state, especially a steam treatment according to EP 0 159 311 or EP 0 519 901 or EP 0 637 451, by a hydrolase treatment according to EP 0 247 998, by a radiation treatment or by a treatment with chemical or chemical/physical methods, e.g. with chaotropic agents, according to WO 94/13329, by a treatment with organic solvents and/or tensides according to EP 0 131 740 or photoinactivation. Nanofiltration also represents a preferred method of depleting viruses within the scope of the present invention.

The pharmaceutical preparation according to the present invention further contains a pharmaceutically acceptable carrier and/or suitable buffer auxiliary preserving and/or stabilizing substances like carbohydrates or salts, or protease inhibitors or cofactors, respectively. The preparation is finally formulated for especially parenteral or topical uses like any known vWF preparation according to the prior art. This may be done by filling it into containers in a form suitable for administration and preferably packing it so as to be storage stable, optionally in the lyophilized or frozen state.

The preparation according to the present invention may be produced both by purification from blood serum or plasma and by a respective expression system. Transgenic animals might as well provide the source of pp-vWF or pro-vWF.

A preparation according to the present invention may also be provided by expression of the pp-vWF or the pro-vWF containing pp-vWF in vivo or ex vivo. Especially suitable for such procedure are cells derived from mammals, in particular human cells, which can be cultured or employed in human gene therapy. Also transformed cells expressing pp-vWF or the pro-vWF as a heterologous protein are a suitable source for obtaining the preparation according to the invention.

A further embodiment according to the present invention is also a pharmaceutical preparation containg a pro-vWF mutant with a

mutation at the cleavage site. Such a mutant has been described by Borchiellini et al. for experimental purposes (Blood 88 (8), 2951-2958 (1996)). The described vWF-Gly 763 has a mutation which provides for a pro-vWF that is unclearable by physiological enzymes. The resistance of the pro-vWF against cleavage leads to the prolonged half-life of the coagulation-promoting activity of the pp-vWF being comprised in the pro-vWF form. Thereby a prolonged action is designed by a specific mutation at the cleavage site.

This specific mutation might by effected by the techniques of Lankhof et al. (Thrombin and Haemostasis 77 (5), 1008 - 13 (1997)) who produced a deletion mutant lacking the A2 domain, which was resistant to proteolysis unless it became sensitive upon unfolding to the molecule.

Other mutant proteins of pp-vWF or pro-vWF that exhibit the properties of the native proteins may also be used for the present preparations. In this case it is preferred to employ an analogue or mutant having at least 80% homology and the function to act as a modified pp-vWF or pro-vWF.

Yet another effect of the present invention is a method for producing a pharmaceutical preparation containing an effective amount of pp-vWF comprising providing a source material containing the vWF propeptide, separating the pp-vWF from the source material and formulating the pp-vWF to a pharmaceutical preparation.

Source material may preferably be blood, serum, blood fractions, colostrum or milk of transgenic animals, or cell culture solutions, especially from cells that have been produced by recombinant DNA-technology. The source material containg pp-vWF preferably contains the pp-vWF in a pro-vWF. Methods and techniques are described in FEBS Letters 351, 345-348 (1994) or Borchiellini et al. supra.

The expression is preferably performed in a way to prevent the processing and maturation of vWF to obtain the pro-vWF. This may

be effected by the omission or inhibition of processing enzymes. The inhibition of processing enzymes like furin or PACE or the multimerase as described in A 770/96 and 769/96 prevents the premature processing of pro-vWF to vWF. On the other hand the pp-vWF might be expressed as a separate protein or obtained upon cleavage and processing of the pro-vWF in vitro.

Yet another preferred embodiment of the present invention is conducting the method by providing a source material containing the pro-vWF as a mutant pro-vWF with a mutation at the cleavage site of the pp-vWF, such as an amino acid change at 763 like pro-vWF-Gly 763.

Alternatively, the pharmaceutical preparation may be produced in the presence of an inhibitor inhibiting the cleavage of the pp-vWF from the pro-vWF. Examples for such inhibitors are antibodies against the cleavage site or a binding peptide directed against the cleavage site or inhibitor of processing enzyme.

As stated above, the preparation and separation steps of pp-vWF are well-known in the art due to various reports of the experiments conducted with pp-vWF (see Isobe et al., Tagaki et al., Leyte et al.).

Of course, the method according to the present invention preferably exhibits a treatment for inactivating or removing viruses, since the pp-vWF is a biological protein and in a form which is administered to humans.

The invention further provides pp-vWF and/or pro-vWF for use as a medicine. The effective dose to elevate the pp-vWF level in vivo to at least twice the physiological amount may be provided by administering the pp-vWF or the pro-vWF once or several times a day. Due to the rather short half-life of the pp-vWF in vivo it might be necessary to administer the protein frequently during the acute disorder.

Yet another aspect of the present invention is the use of pp-vWF

and/or pro-vWF containing the pp-vWF for the preparation of a pharmaceutical composition for treating a patient at a risk of blood coagulation disorders, such as vWD, hemophilia (f.e. phenotypic hemophilia, hemophilia A and factor VIII inhibitors).

The effective dosage of the preparation when applied will vary depending on the respective syndrom and preferably should be chosen after determination of the blood levels of the critical blood factors or risk for thrombosis in the patient. The optimum dosage also depends on whether or not the parenteral, preferably intravenous, subcutaneous or intramuscular adiministration is to be effected in bolus form or as a depot. By using a suitable carrier material such as liposomes a peroral administration is feasible. It also depends on whether it is to be applied systemically and/or locally at the site of the blood coagulation disorder.

Therefore, the invention also provides for a method of treating a patient at a risk of blood coagulation disorders comprising administering to said patient an effective amount of vWF-propeptide or pro-vWF. Preferably, a patient suffering from vWD, phenotypic hemophilia, hemophilia A or factor VIII inhibitors is treated according to the invention.

Due to the positive properties pp-vWF or pro-vWF exhibit when combined with preparations with a risk for arterial thrombosis, such as vWF-preparations, it is another aspect of the present invention to use pp-vWF or pro-vWF to reduce thrombosis risk in vWF-preparations. Thereby the potential exaggeration of arterial thrombus formation is effectively down-modulated, whereas the intrinsic and extrinsic blood coagulation is promoted in case of a coagulation deficiency.

In particular the compatibility of a vWF preparation is ameliorated and improved by the addition of and combination with the pp-vWF or pro-vWF in effective amounts. Because of the controlling function of the vWF propeptide it further contributes to the treatment and prevention of adverse reactions of endogenous and exogenous vWF, particularly elevated vWF

levels in patients associated with thrombotic thrombocytopenic purpura, Henoch Schönlein purpura, preclampsia, neonatal thrombocytopenia or hemolytic uremic syndrome, myocardial infarction or a poor outcome following arterial surgery.

The present invention will be explained in more detail by way of the following examples and drawing figures to which, however, it shall not be restricted.

Fig.1 shows the effect of pro-vWF on the thrombin generation in plasma in the presence of platelets,

Fig.2 shows the dose dependent effect of pro-vWF on the thrombin generation in plasma in the presence of platelets,

Figs.3a and 3b show the comparison of the in vivo effect of pro-vWF and plasma derived vWF in a dog.

Examples:

1. The effect of provWF an ppvWF on the thrombin generation in plasma in the presence of platelets.

Severe vWD plasma (George King Bio-Medical Inc., USA) which was previously reconstituted with 1 U/ml FVIIIC (Recombinate, Baxter, USA) (200 µl) was incubated with 50 µl washed platelets from a severe vWD patient (Type III) in the presence of 0.1 U/ml FEIBA (Immuno, Austria) and 16 mM $CaCl_2$. Alternatively FVIIa could also be used as an activator, preferably in a final concentration of 0.2 µg/ml. Subsamples of 10 µl were withdrawn at time intervals, and added to 300 µl chromogenic substrate for thrombin (D-cyclohexyl-gly-L-Ala-L-Arg -pNA; Immuno, Austria) containing 3 mM EDTA to stop any further reactions. The reaction was stopped by the addition of 100 µl of 75 % (v/v) acetic acid, and the absorbance, which is the function of the thrombin concentration, was measured at 405 nm.

Two different vWF preparations (containing lower and higher amounts of provWF and propeptide) were added to the factor VIII

reconstituted plasma samples in a final concentration of 1 Risto U/ml, and thrombin generation was followed. (1 Risto U/ml = the ristocetin cofactor activity of the vWF in normal plasma). Thus the plasma samples contained in the case of the preparation with the low provWF and low propeptide 0.24 nM provWF and 0.19 nM propeptide, while the other one contained 34 nM provWF and 4 nM propeptide, respectively.

The results are depicted on Figure 1.

It is clear from the curves, that albeit both preparations increased the rate of thrombin generation, the high provWF and high propeptide containing product had a stronger effect and produced the enhanced thrombin generation.

2. The effect of provWF on the thrombin generation in plasma in the presence of platelets.

The effect of a recombinant vWF preparation, which contained more then 90% provWF of the total vWF antigen on the thrombin generation was investigated in the system described by the Example 1. Washed platelets from an other severe vWD patient (Type III) and the isolated plasma sample of the same patient, reconstituted with 1 U/ml FVIIIC were used in the experiments. Recombinant provWF of 2 and 5 vWF-Ag U/ml (1 Ag U = the vWF antigen amount in 1 ml normal plasma) respectively, were added to the plasma samples and thrombin generation was compared with that of in the absence of vWF. Figure 2 shows, that above a threshold of 2 vWF-Ag U/ml an increased thrombin generation was observed.

Example 3. Comparison of the in vivo effect of a high provWF containing recombinant and a plasma derived vWF preparation.

A vWF deficient dog (vWF antigen below the detection limit, and factor VIII activity about 50 % of the normal) was anesthetized and infused with 35 Risto U/ml of the recombinant vWF used in the example 2, containing more then 90 % of provWF. Prior to the infusion and 15, 30, 40 minutes, 1, 2, 3, 6, 24, 48, 72, an 95

hours post infusion plasma samples were taken. 95 hours later a plasma derived vWF preparation (Haemate HS - Behringwerke, Marburg, Germany) was added, and plasma samples were taken again at the same intervals after the infusion, as before. This plasma derived vWF preparation further contained Factor VIII:C, but no provWF and no ppvWF. The plasma samples were analyzed for total vWF antigen (Asserachrom ELISA, Boehringer), provWF and propeptide antigen (according to Borchiellini at al Blood 88, 2951, 1996), as well as for the thrombin generation potential, as described in Example 1. The thrombin potential is defined as the maximum thrombin concentration measured as described in Example 1.

Figure 3 shows the correlations of the various parameters, expressed as percent of maximum, regarding the initial values as zero.

Thrombin potential increased in parallel with the increase of propeptide after the treatment with a recombinant provWF preparation. ELISA results showed, that a few percent of provWF remained in the circulation after 15 minutes, and it could no longer be detected (data not shown), but a significant increase in the propeptide and vWF was observed. In contrast, no propeptide and also no substantial thrombin potential was observed in the dog after the plasma derived vWF infusion, despite of the vWF antigen level increase.

Claims:

1. Pharmaceutical preparation for treating blood coagulation disorders comprising an effective amount of vWF propeptide.

2. Preparation according to claim 1 essentially consisting of vWF propeptide.

3. Preparation according to claim 1 comprising pro-vWF containing the vWF propeptide.

4. Preparation according to claim 3, wherein the pro-vWF is a recombinant pro-vWF.

5. Preparation according to claim 1 to 4 which further contains a hemostasis protein, preferably a blood factor.

6. Preparation according to claim 5, wherein the blood factor is selected from the group consisting of mature vWF, factor VIII, activated blood coagulation factors, blood factors with FEIB-activity and FEIBA.

7. Preparation according to claim 6, wherein the pro-vWF is complexed to factor VIII.

8. Preparation according to claim 1 to 7 which further contains a platelet component, preferably at least one of a collagen, a platelet glycoprotein, a platelet, fibrinogen, fibrin, heparin, or a derivative thereof.

9. Preparation according to claim 1 to 8 which further contains phospholipids.

10. Preparation according to claim 1 to 9 which is treated for virus inactivation or virus removal.

11. Preparation according to claim 1 to 10 which further contains a pharmaceutically acceptable carrier.

12. Preparation according to claim 1 to 11, wherein the vWF propeptide is recombinant vWF propeptide.

13. A method for producing a pharmaceutical preparation containing an effective amount of vWF propeptide comprising providing a source material containing the vWF propeptide, separating the vWF propeptide from the source material and formulating the vWF propeptide to a pharmaceutical preparation.

14. A method according to claim 13, wherein the vWF propeptide is treated for inactivating or removing viruses.

15. A method accoding to claim 13 or 14, wherein the source material is plasma or a plasma fraction.

16. A method according to claim 13 or 14, wherein the source material is obtained from a cell culture.

17. A method according to claim 13, wherein the vWF propeptide is produced by recombinant DNA technology.

18. A method according to claim 13 to 17, wherein the vWF propeptide is contained in a pro-vWF.

19. A method according to claim 18, wherein the pro-vWF is a mutant pro-vWF with a mutation at the cleavage site of the vWF propeptide.

20. A method according to claim 13 to 19, wherein the pharmaceutical preparation is produced in the presence of an inhibitor which inhibits the cleavage of the vWF propeptide from the pro-vWF.

21. A method according to claim 13 to 20, wherein the vWF propeptide is separated by chromatography, preferably by affinity chromatography, from the source material.

22. A method according to claim 21, wherein the affinity chromatography employs carrier materials with ligands specific

for the vWF propeptide.

23. vWF-propeptide as a medicine.

24. pro-vWF containing vWF propeptide as medicine.

25. Use of vWF propeptide and/or pro-vWF containing the vWF propeptide for the preparation of a pharmaceutical composition for treating a patient at a risk of blood coagulation disorders.

26. Use of vWF propeptide and/or pro-vWF composition for the preparation of a pharmaceutical composition for the treatment of vWD inhibitors.

27. Use of pp-vWF or pro-vWF for improving the compatibility of pharmaceutical vWF-preparations.

28. Use of pp-vWF or pro-VWF for the preparation of a pharmaceutical composition for the treatment or prevention of adverse effects of endogenous or exogenous vWF, particularly elevated vWF levels associated with thrombotic thrombocytopenic purpura, Henoch-Schönlein Purpura, preclampsia, neonatal thrombocytopenia or hemolytic-uremic syndrome, myocardial infarction or poor outcome following arterial surgery.

29. Use of vWF propeptide and/or pro-vWF composition for the preparation of a pharmaceutical composition for the treatment of hemophilia.

30. Use of vWF propeptide and/or pro-vWF composition according to claim 29 for the treatment of phenotypic hemophilia, hemophilia A and factor VIII inhibitors.

Summary

**Pharmaceutical Preparation Comprising vWF Propeptide**

Described is a pharmaceutical preparation for treating blood coagulation disorders comprising an effective amount of vWF propeptide as well as a method for producing such a preparation.

Fig 1: The effect of provWF on the thrombin generation in plasma in the presence of platelets.

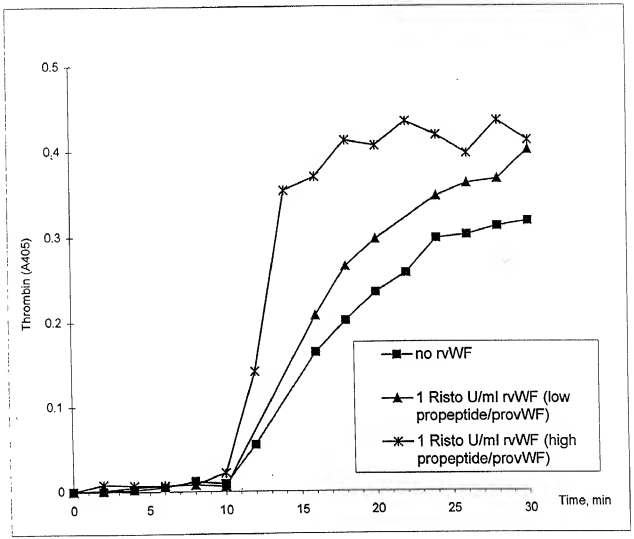


Fig 2: Dose dependent effect of provWF on the thrombin generation in plasma in the presence of platelets.

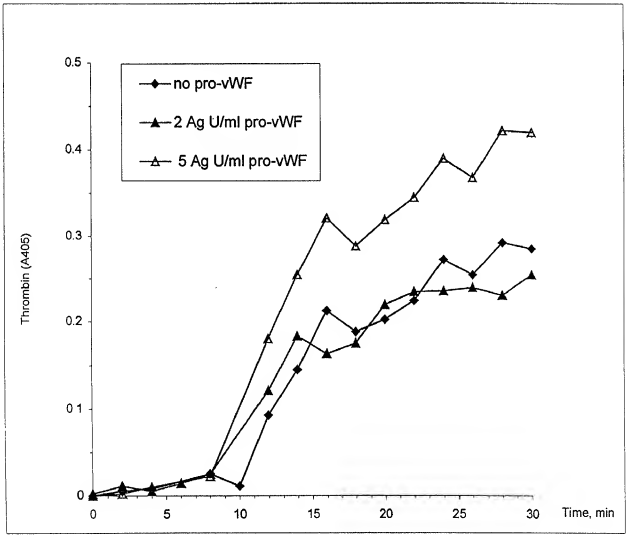
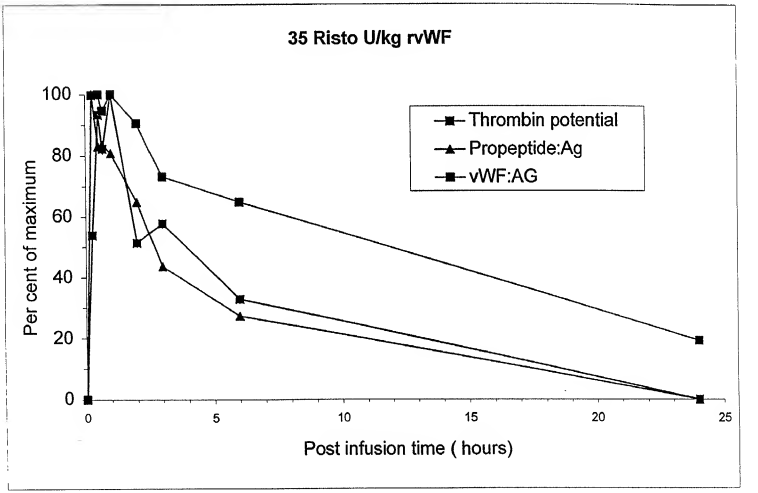


**Fig. 3a Comparison of the in vivo effect a recombinant vWF (pro-vWF) and plasma derived vWF preparation in a dog**



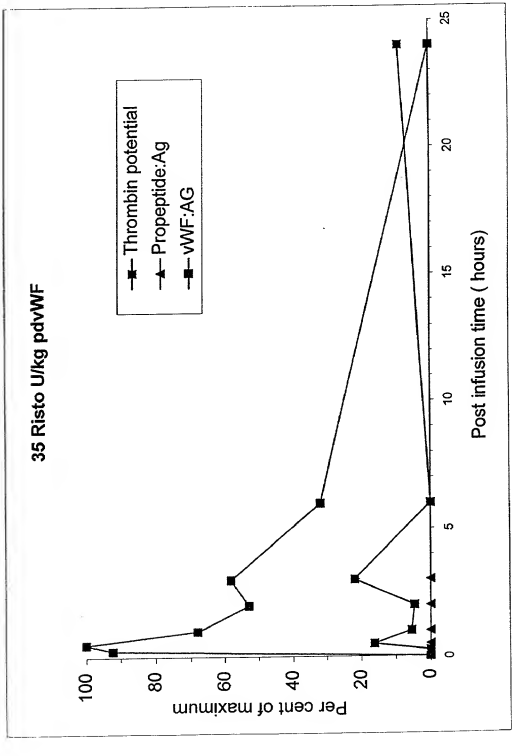

35 Risto U/kg pdvWF
Thrombin potential
Propeptide:Ag
vWF:AG
Per cent of maximum
Post infusion time ( hours)
0
20
40
60
80
100
0
5
10
15
20
25

Fig.3b

**DECLARATION FOR PATENT APPLICATION** Docket Number: BHV-314.01

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PHARMACEUTICAL PREPARATION COMPRISING VWF PROPEPTIDE

the specification of which (check one): ( ) is attached hereto.
(X) was filed on 05/26/98 as ~~United States~~ International Patent Application Number PCT/EP98/03090, and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulation, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

| Prior Foreign Application(s) | | | Priority Claimed |
|---|---|---|---|
| A 917/97 (Number) | AUSTRIA (Country) | 28/May/1997 (Day/Month/Year Filed) | (X) Yes ( ) No |
| _____ (Number) | _____ (Country) | _____ (Day/Month/Year Filed) | ( ) Yes ( ) No |

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States Provisional application(s) listed below.

_____ (Application Number) _____ (Filing Date)

_____ (Application Number) _____ (Filing Date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

| | | |
|---|---|---|
| _____ (Application Number) | _____ (Filing Date) | _____ (Status: patent, pending, abandoned) |
| _____ (Application Number) | _____ (Filing Date) | _____ (Status: patent, pending, abandoned) |

I hereby appoint Beth E. Arnold, Reg. No. 35,430; Paula Campbell, Reg. No. 32,503, Charles H. Cella, Reg. No. 38,099; Isabelle M. Clauss, Reg. *(see attached)*; Edward J. Kelly, Reg. No. 38,936; Donald W. Muirhead, Reg. No. 33,978; Chinh Pham, Reg. No. 39,329; Diana Steel, Reg. No. 43,153, Matthew P. Vincent, Reg. No. 36,709; and Anita Varma, Reg. No. 43,221 as attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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